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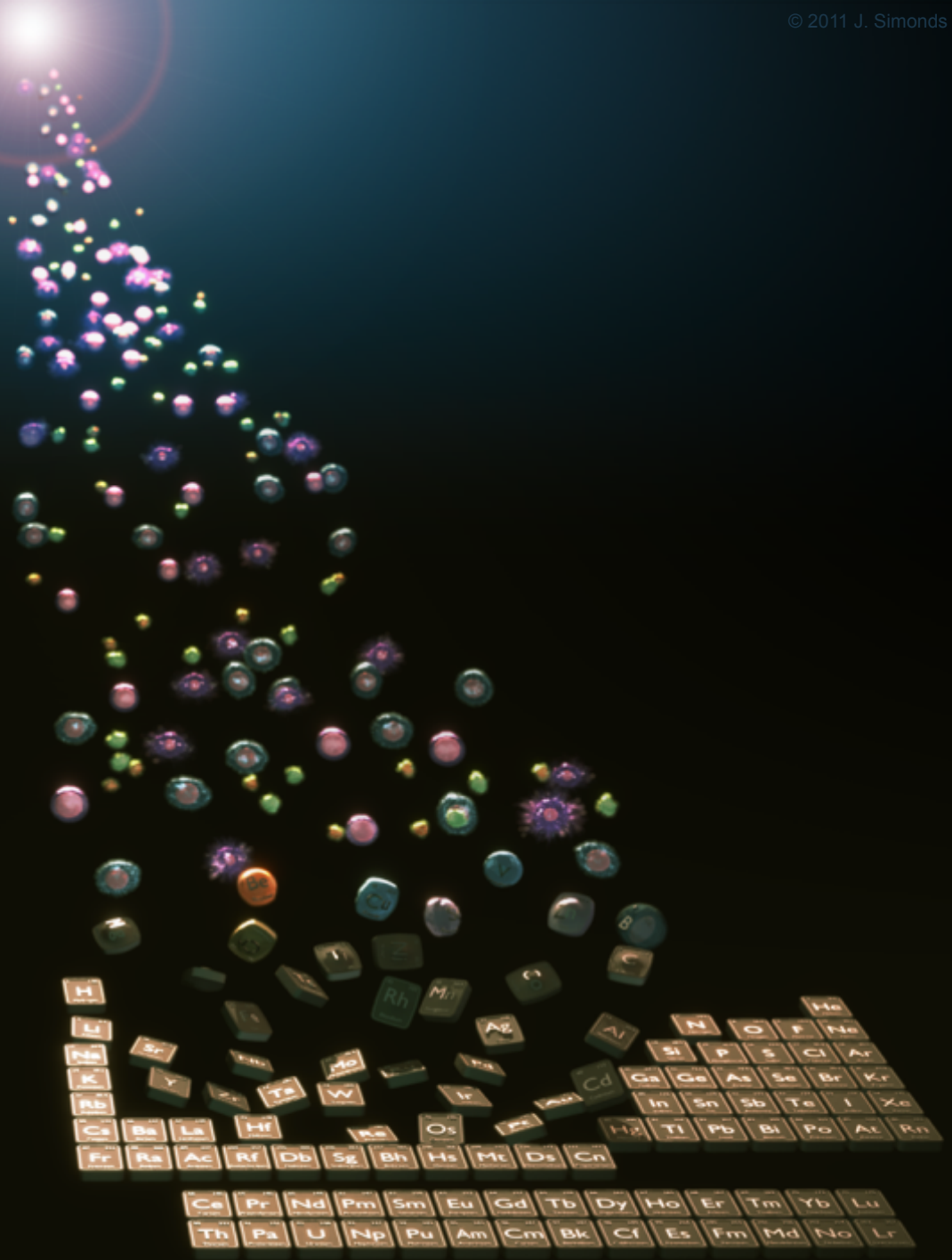
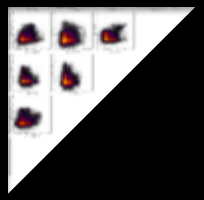
Tools and approaches for mass cytometry data analysis

Erin Simonds, PhD
W. A. Weiss Lab, UCSF

May 23, 2013



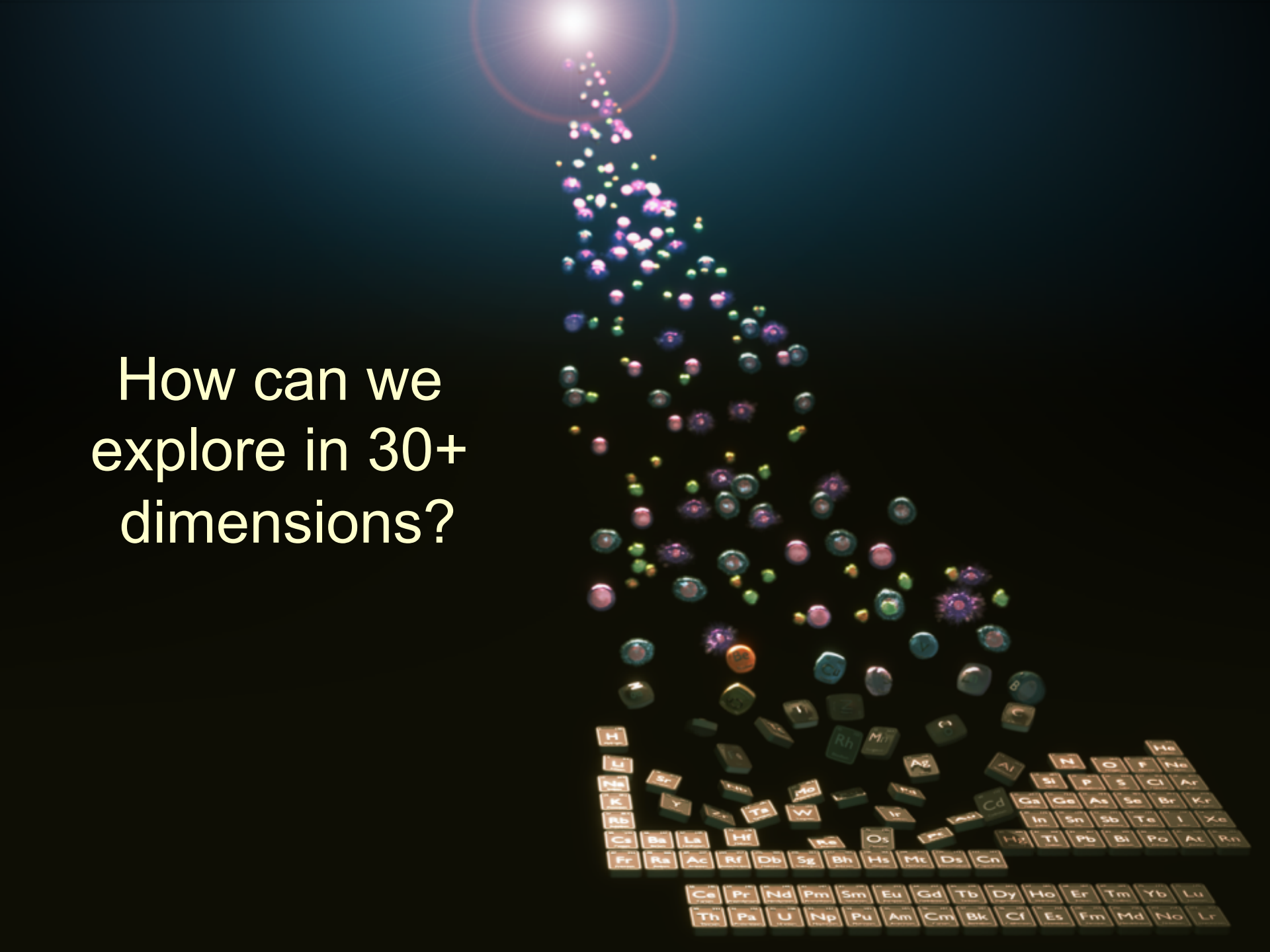
Biaxial plots are not a scalable solution



Parameters: 32

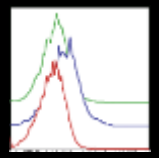
Plots: 496

How can we explore in 30+ dimensions?

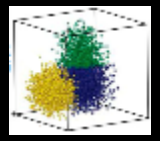


The cytometrist's toolbox

Transform raw data

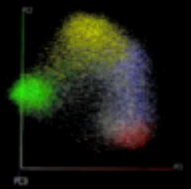

Histogram

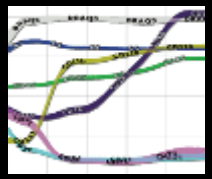
Biaxial plot


3D plot

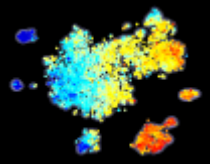
Radar

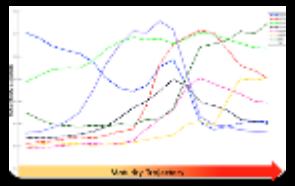
Reduce dimensionality


PCA

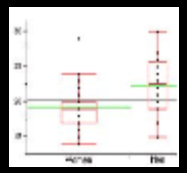

Gemstone

SPADE


viSNE

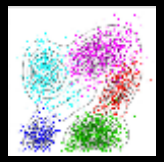

Wanderlust

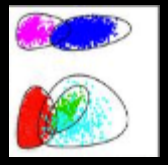
Summarize statistics

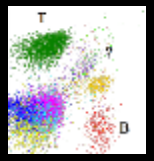

Box plot

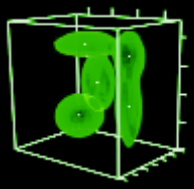
Heatmap

Identify clusters


FlowClust


FlowMerge


FLOCK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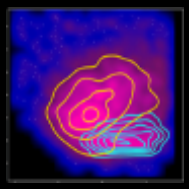
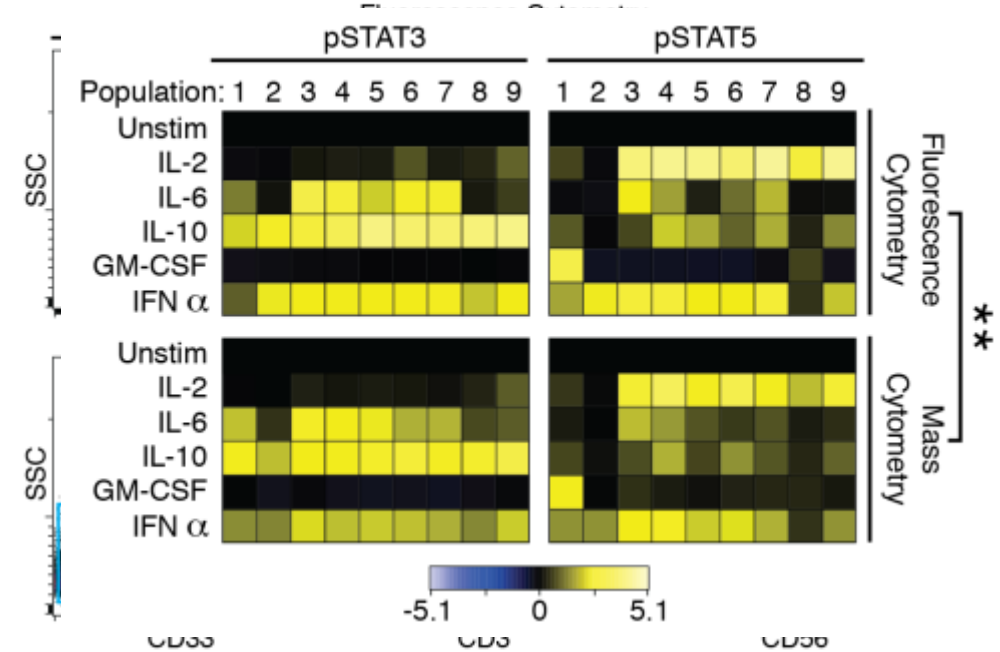

SamSPECTRAL

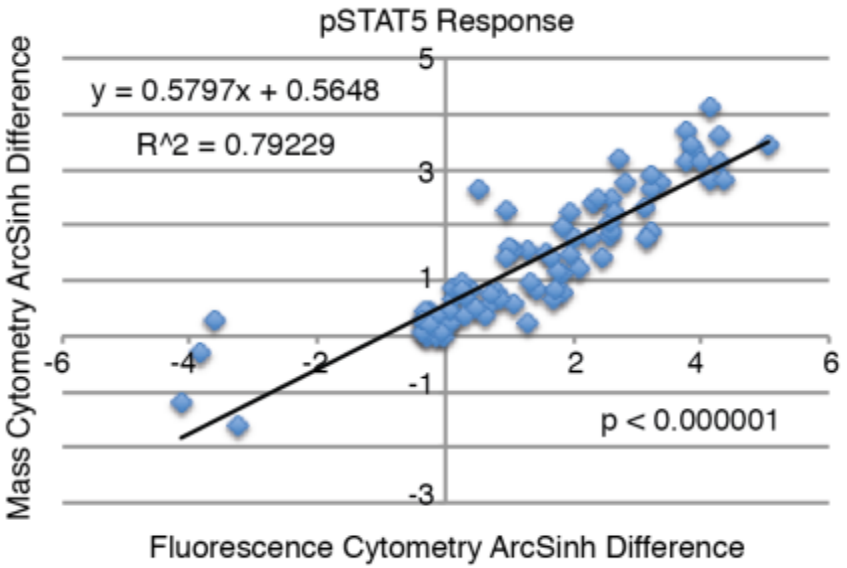
Vetting the CyTOF: In a fair fight, nearly identical data

Experimental design

7 Surface Markers (CD 3, 4, 8, 20, 33, 45RA, 56)

2 Functional Markers (pSTAT3 & 5)





Bendall, Simonds, et al. *Science,* May 2011

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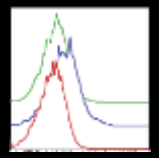
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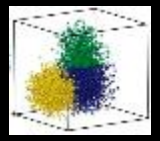
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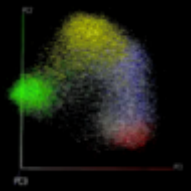
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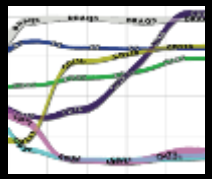
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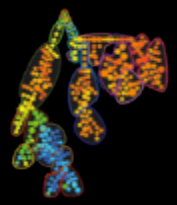
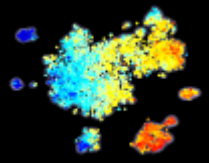

SPADE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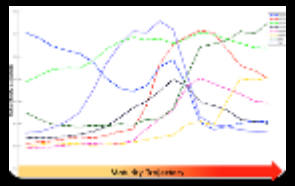
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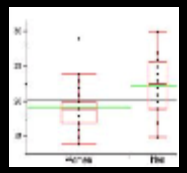
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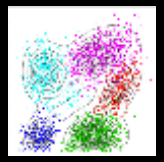
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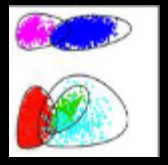
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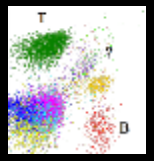
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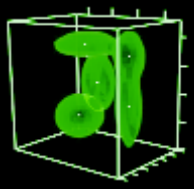
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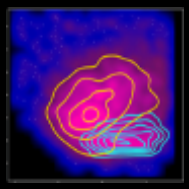
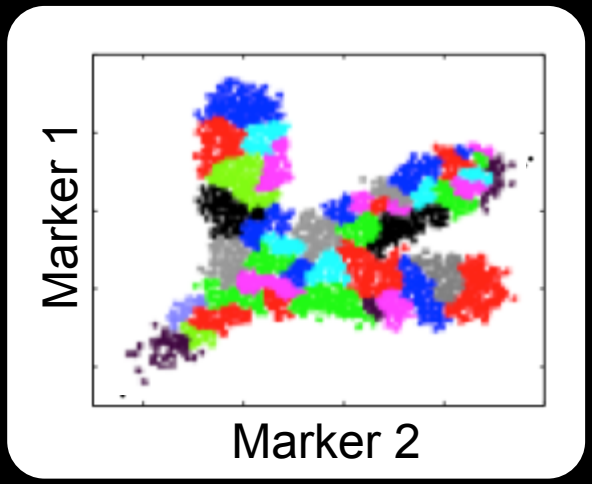
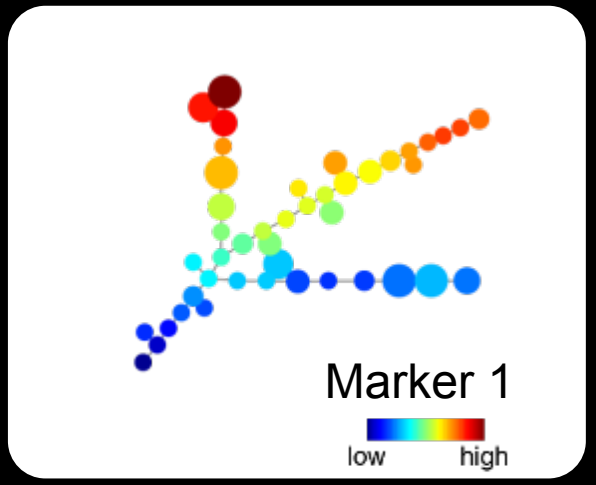
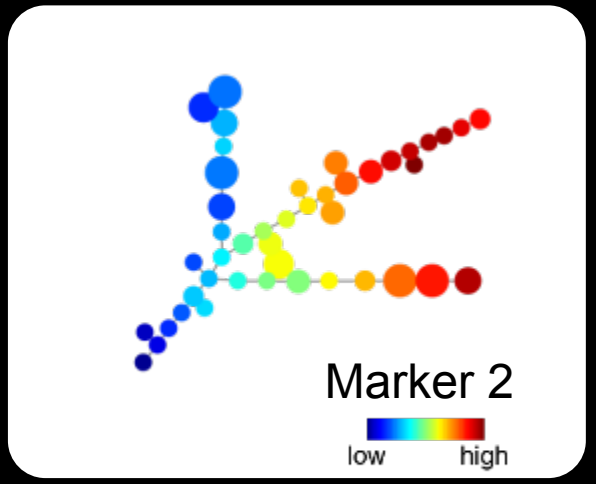

SamSPECTRAL

SPADE: Spanning-tree Progression Analysis of Density-normalized Events

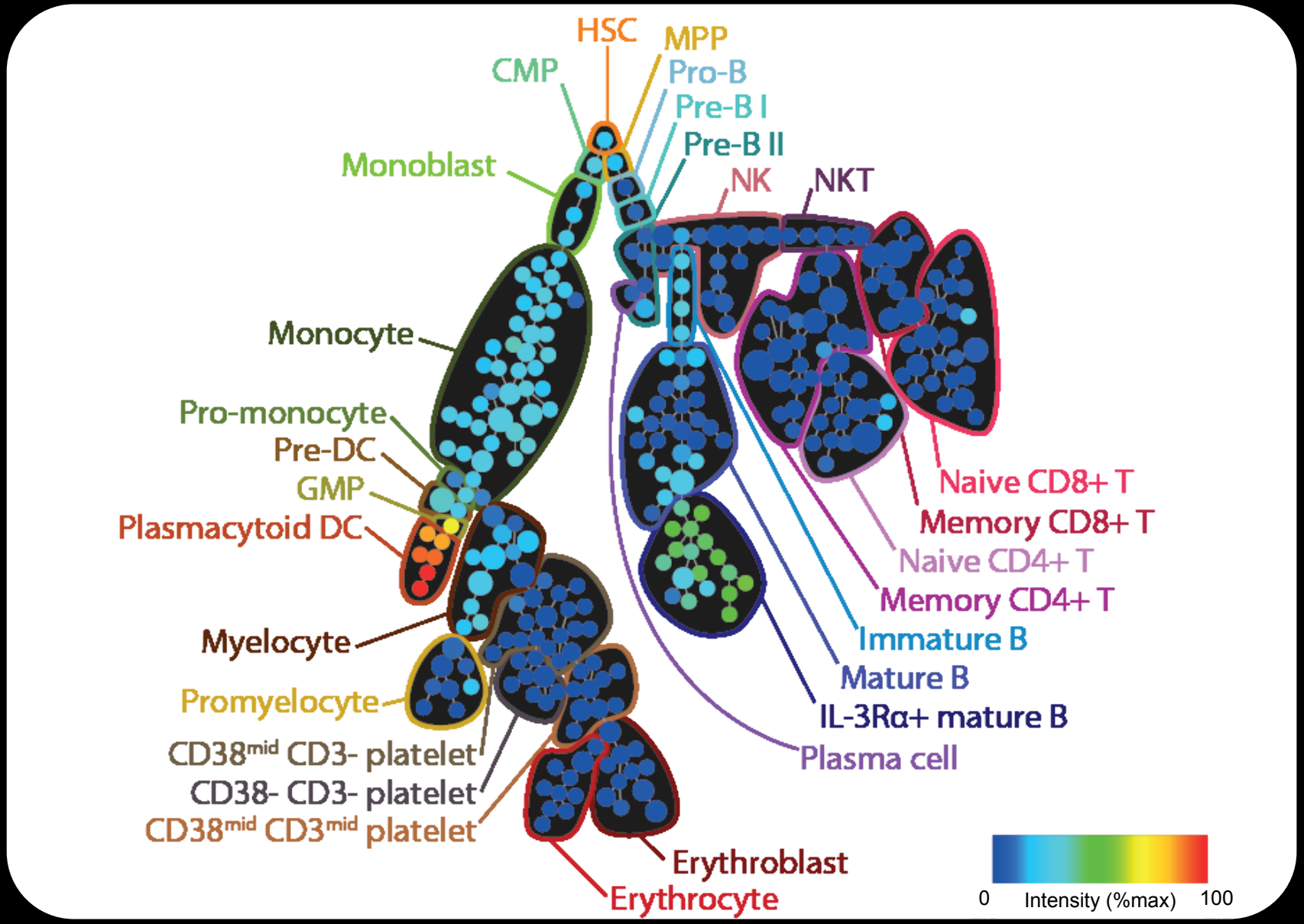






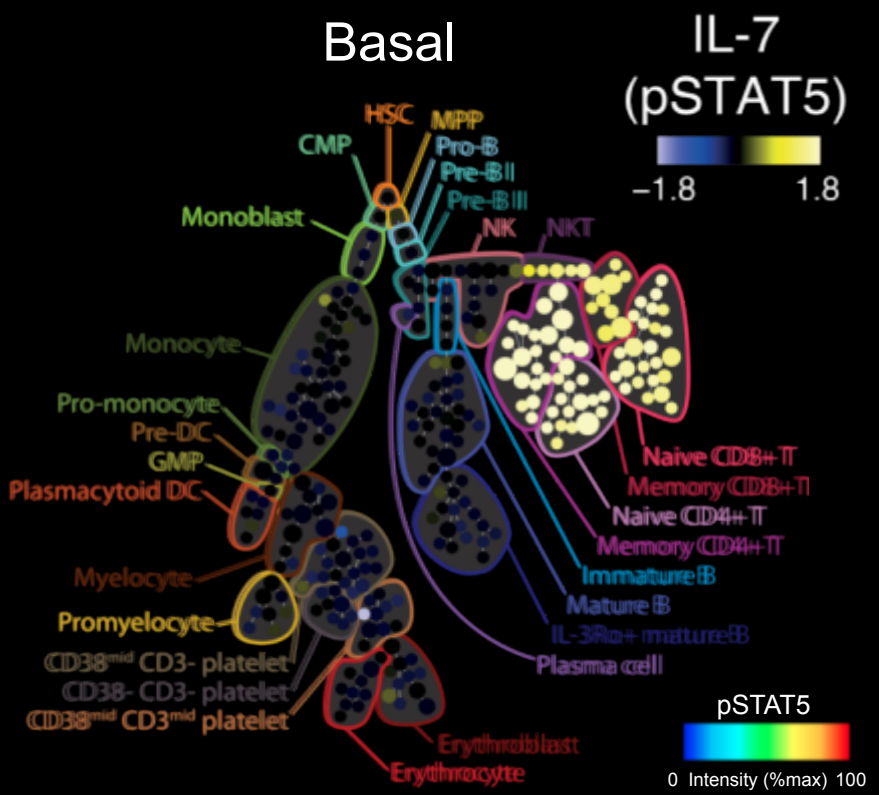
Qiu et al., *Nature Biotechnology*, Oct. 2011

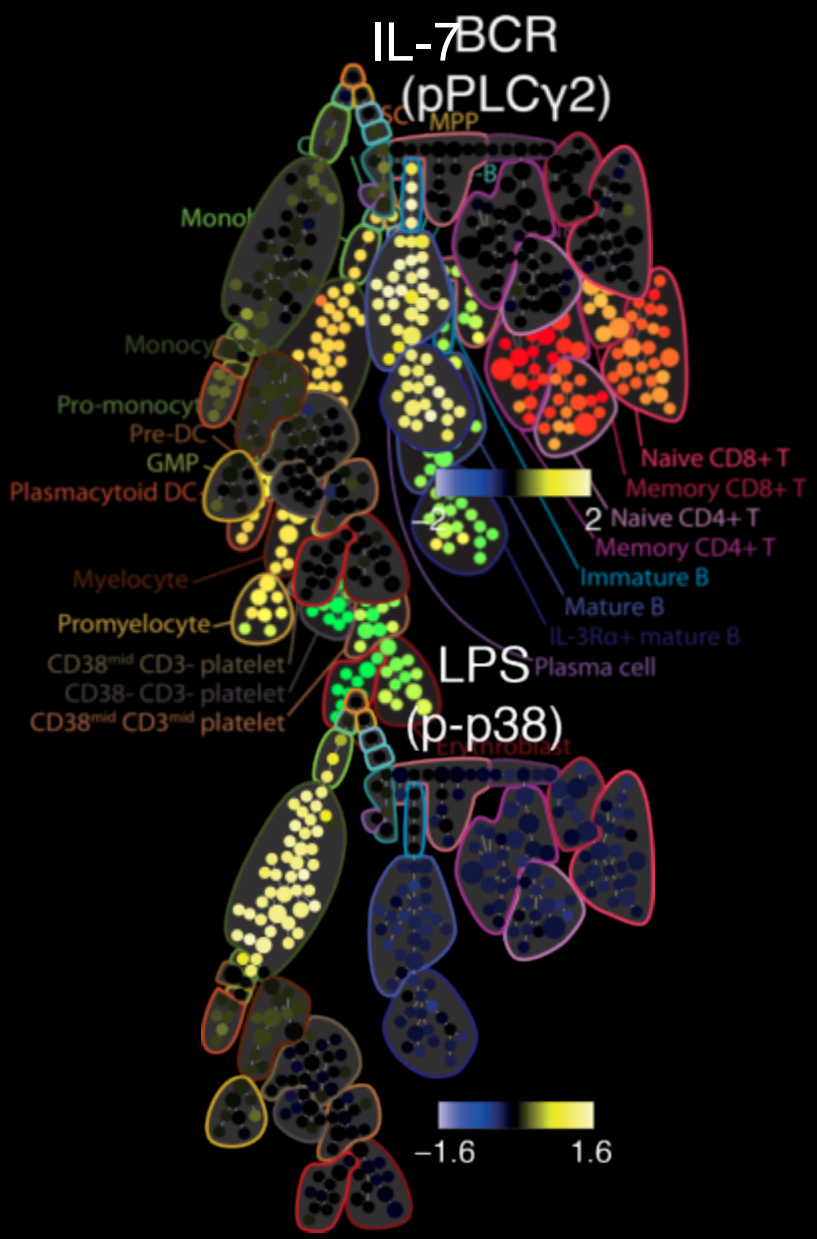
SPADE projects bone marrow as a continuum of phenotypes



Bendall, Simonds, et al. *Science*, May 2011

Rediscovery of canonical signaling pathways





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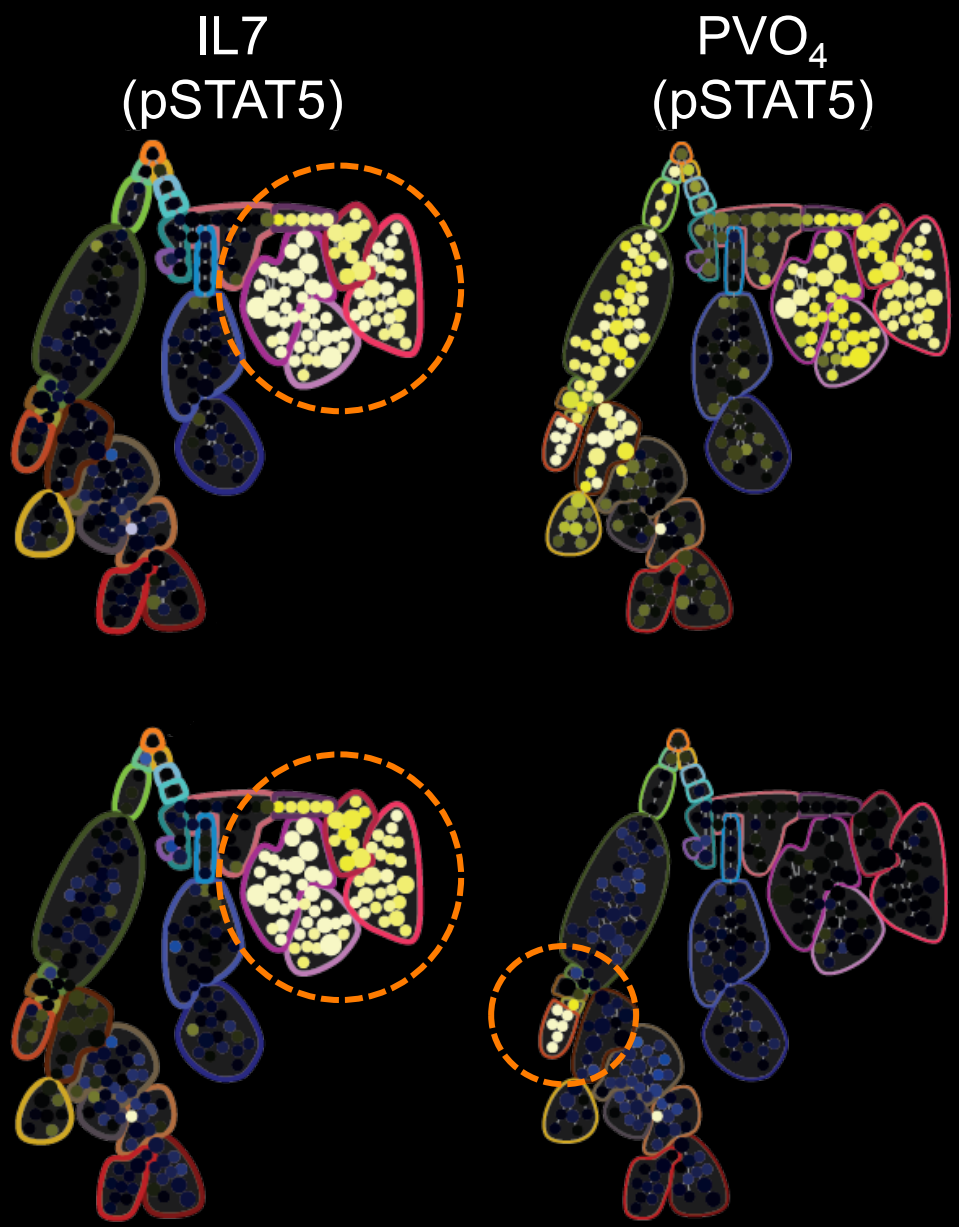
Dasatinib differentially affects immune cell subsets



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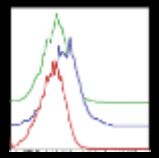
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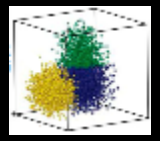
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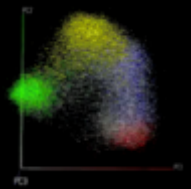
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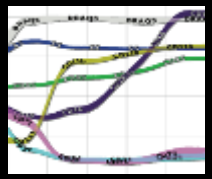
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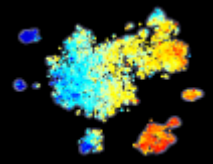
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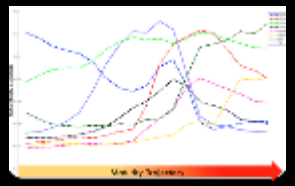
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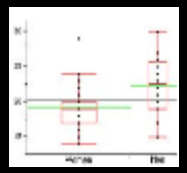
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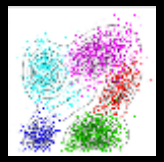
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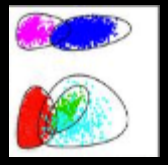
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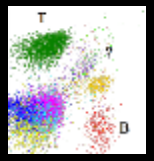
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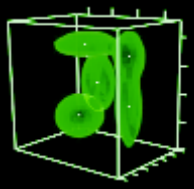
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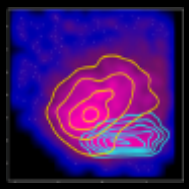
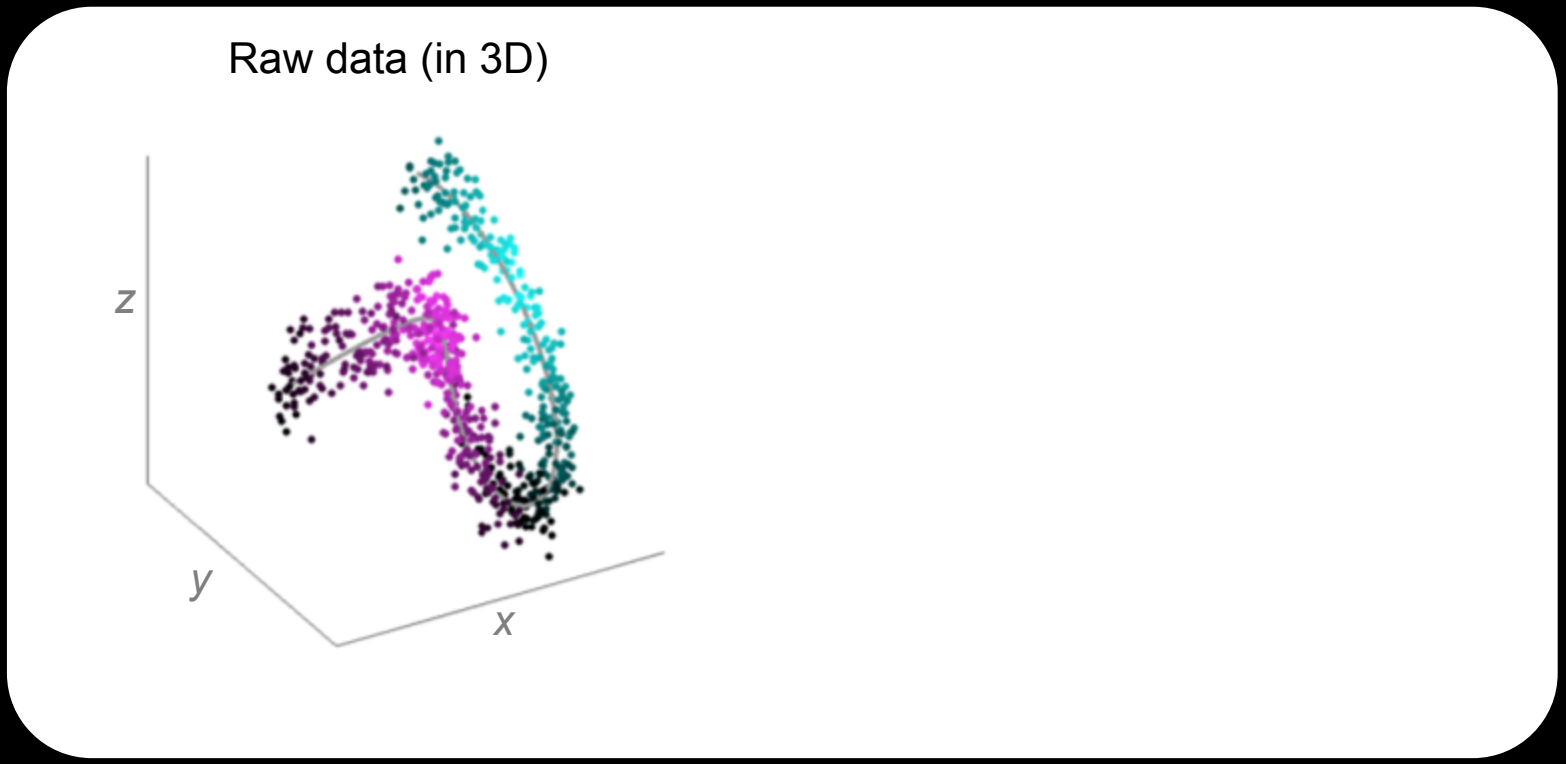
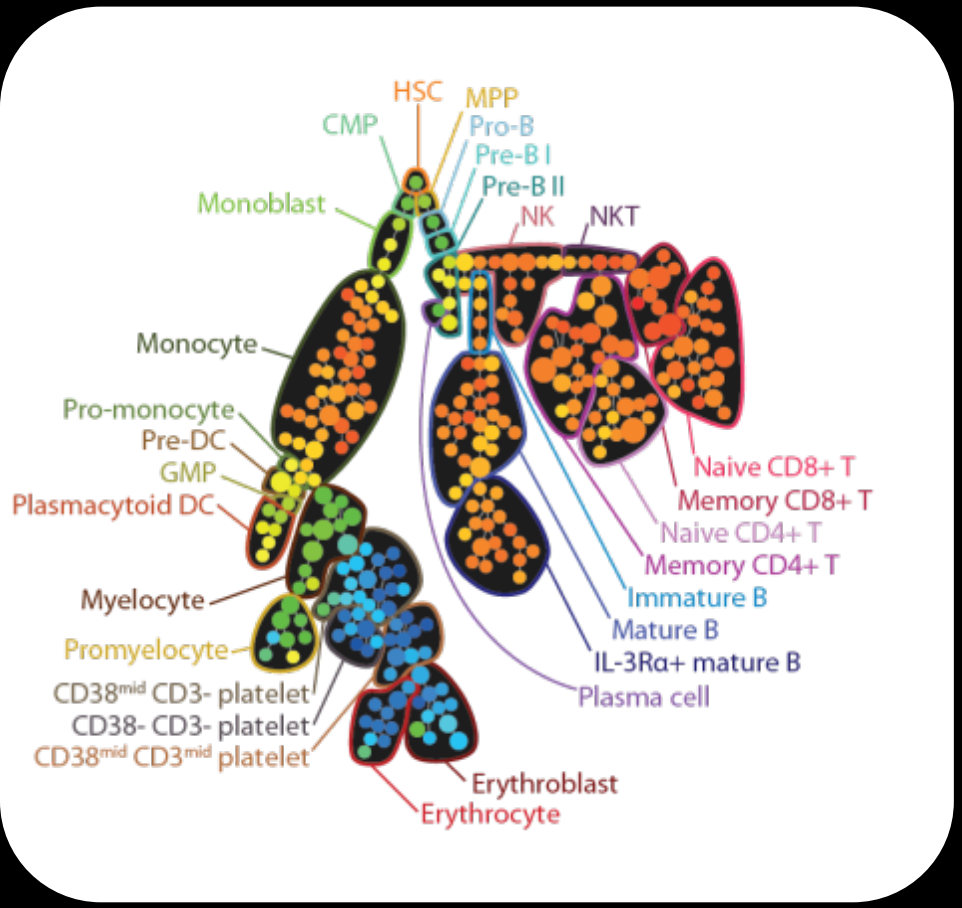

SamSPECTRAL

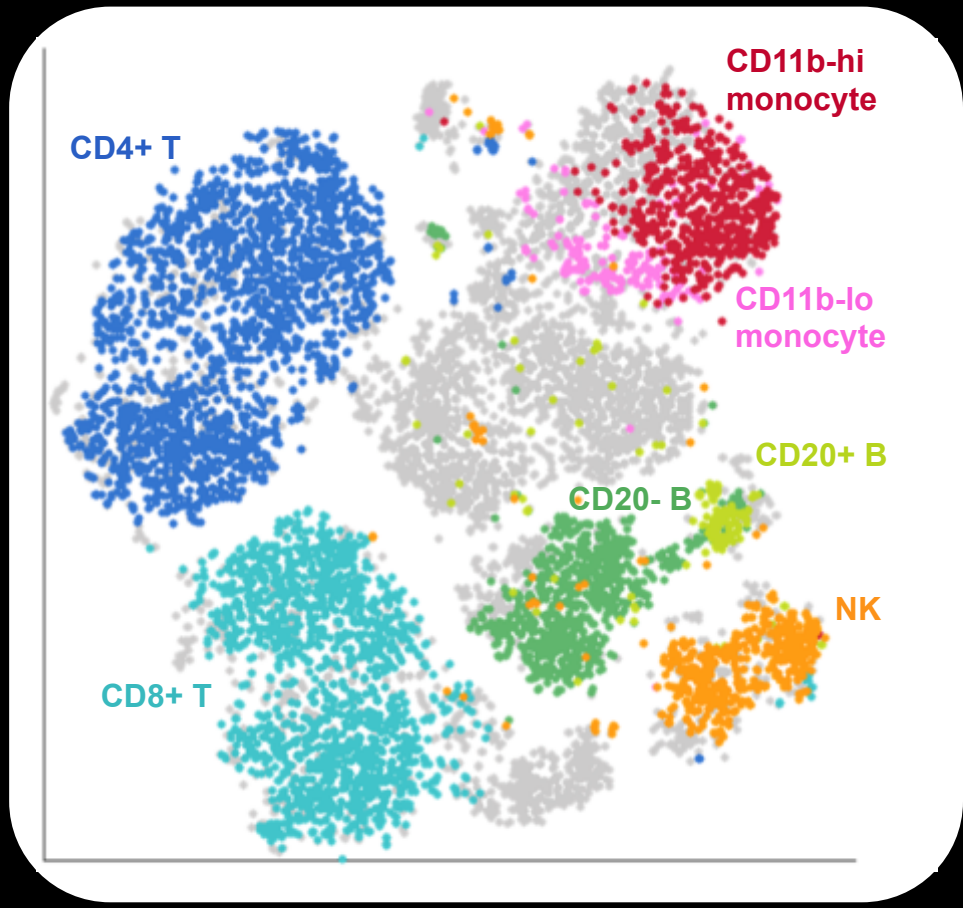
viSNE preserves high-dimensional, non-linear relationships



Amir et al., *Nature Biotechnol.* ePub 19 May 2013

viSNE maps healthy marrow as discrete populations



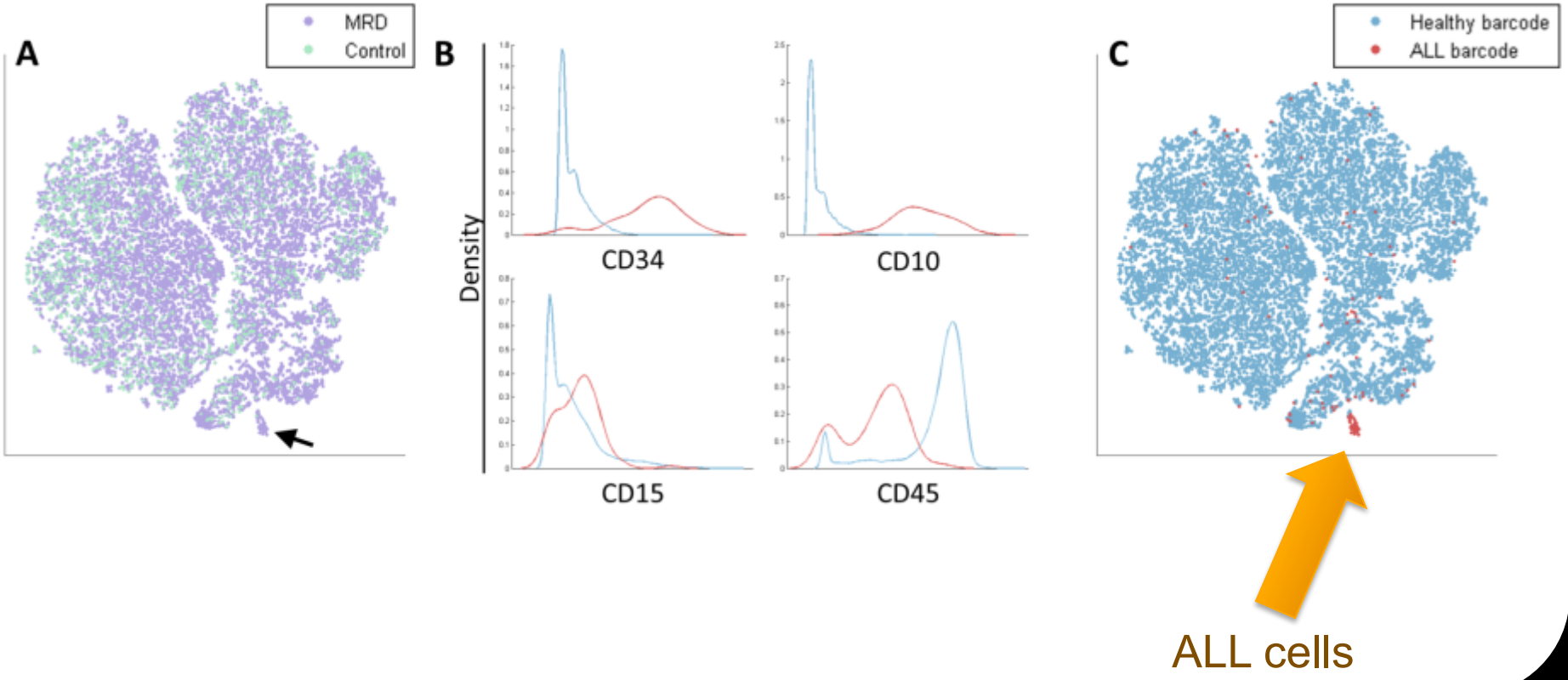


Amir et al., *Nature Biotechnol.* ePub 19 May 2013

viSNE can help detect rare subpopulations

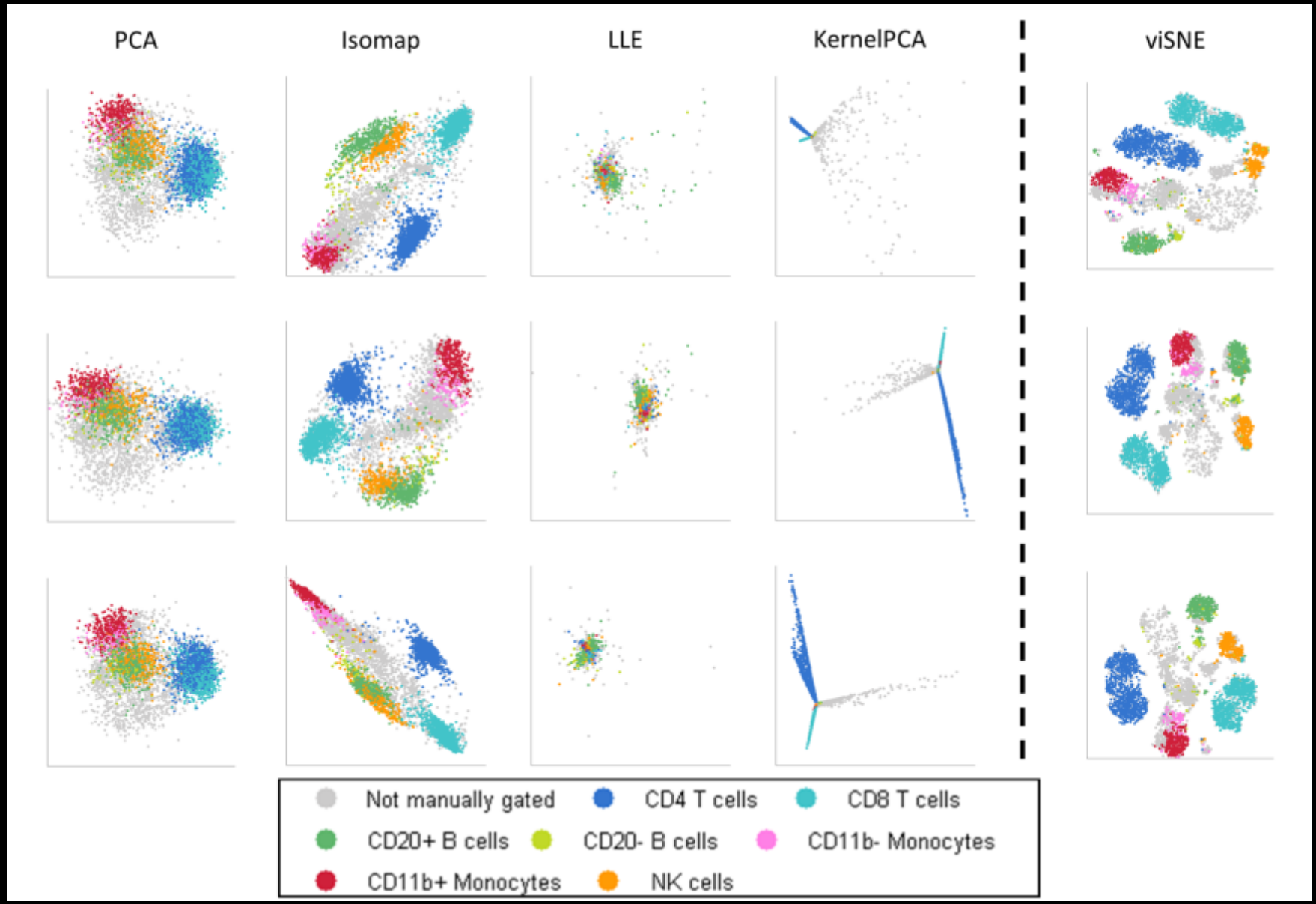
Barcode A: Healthy bone marrow cells

Barcode B: ALL cells spiked into sample at 0.25% frequency (1/ 400 cells)



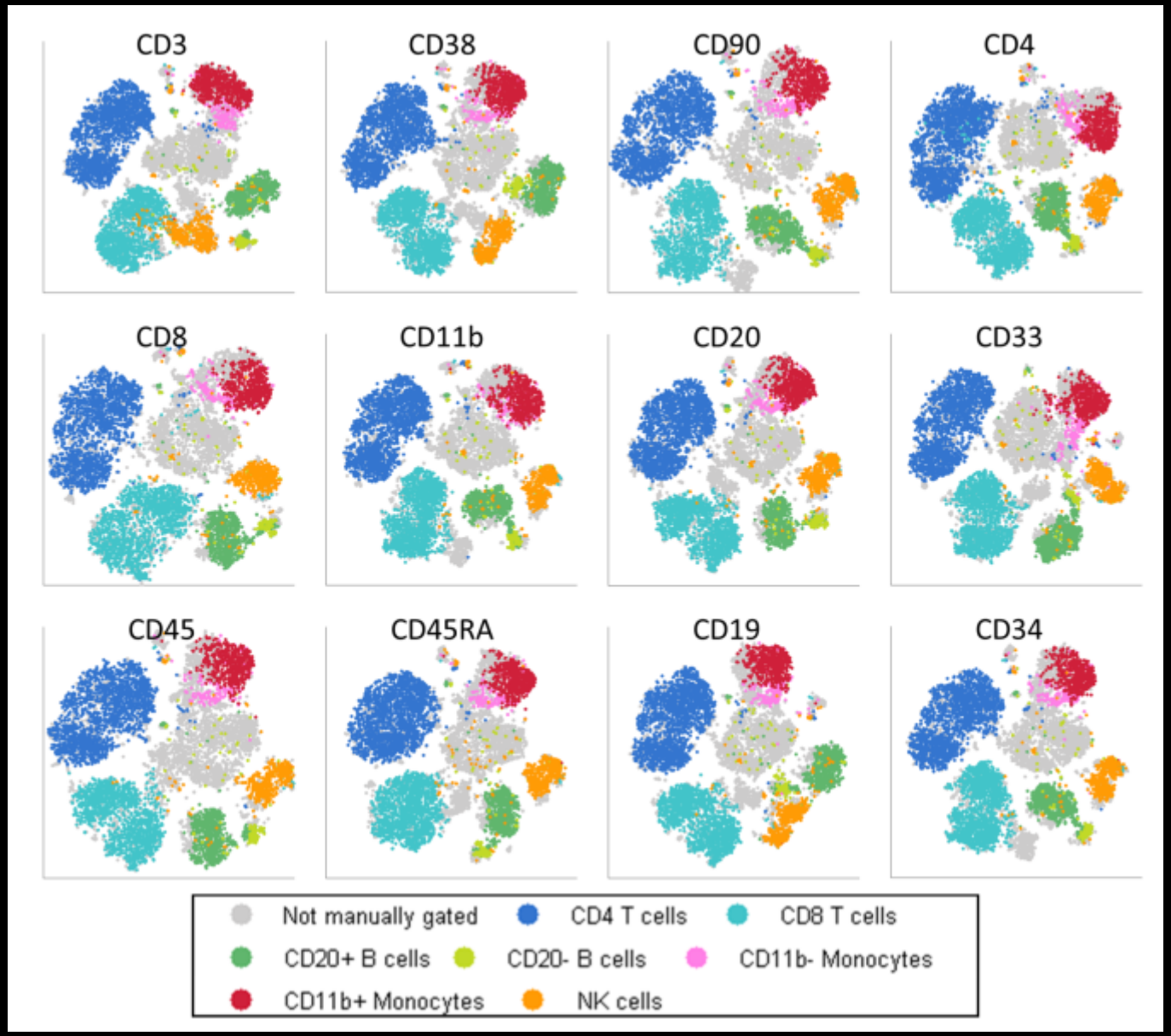
Amir et al., *Nature Biotechnol.* ePub 19 May 2013

Same data, different dimensionality reduction algorithms

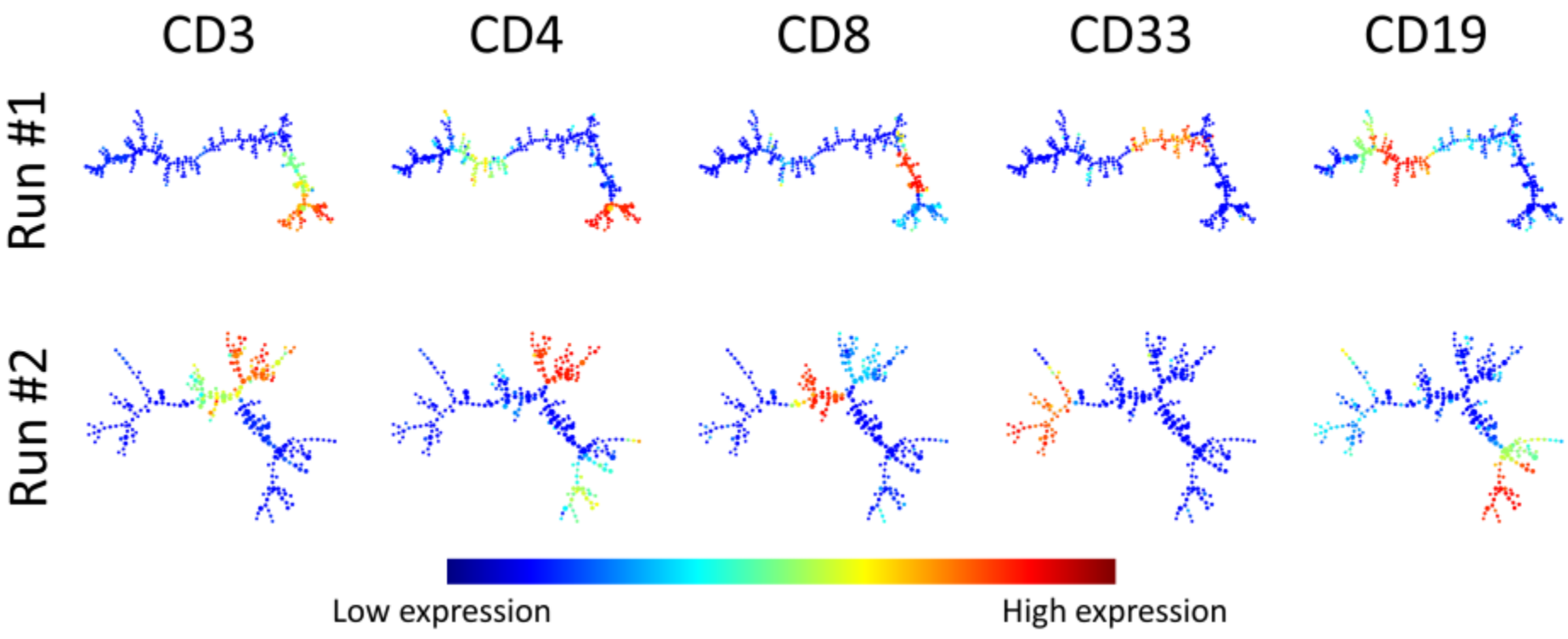


Amir et al., *Nature Biotechnol.* ePub 19 May 2013

viSNE is still stochastic, but much more reproducible



SPADE's stochasticity causes difference between runs



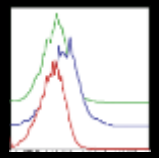
So why should I ever use SPADE?

→ Clustering allows *comparison* between samples

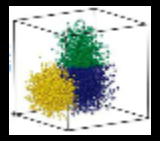
Amir et al., *Nature Biotechnol.* ePub 19 May 2013

The cytometrist's toolbox

Transform raw data

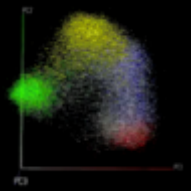

Histogram

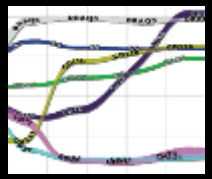
Biaxial plot


3D plot

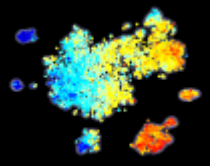
Radar

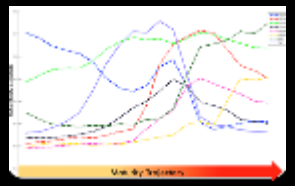
Reduce dimensionality


PCA

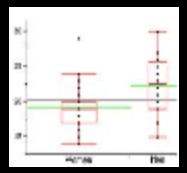

Gemstone

SPADE


viSNE


Wanderlust

Summarize statistics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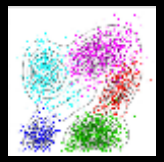
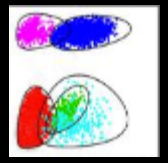
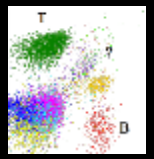
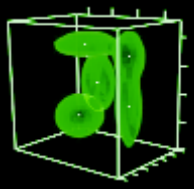
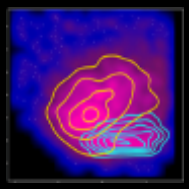
Identify clusters


FlowClust

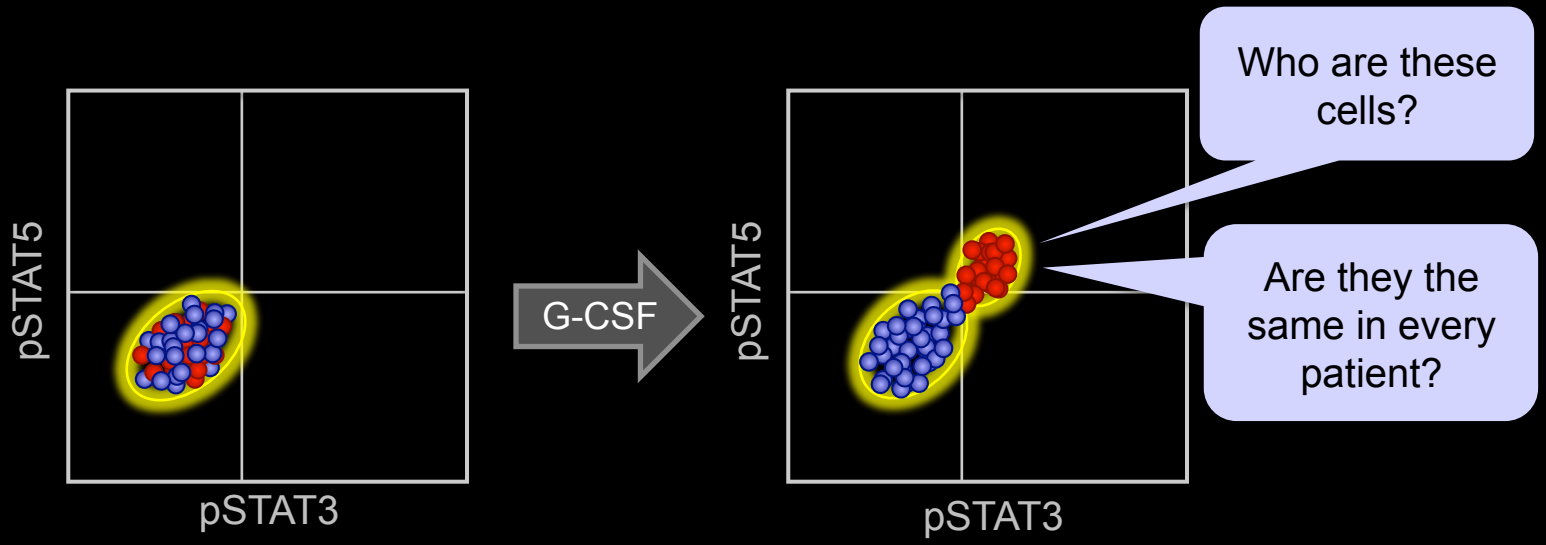

FlowMerge


FLOCK

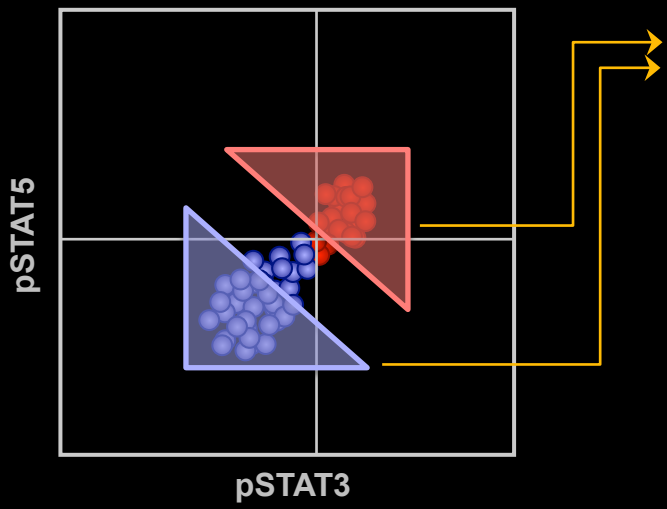

FLAME


SamSPECTRAL

Goal: Characterize the GCSF-responsive subpopulation



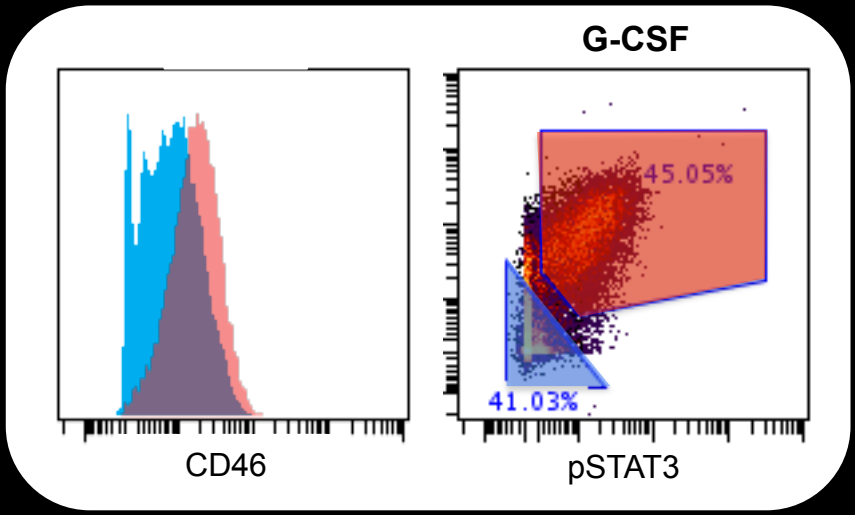
Surface marker profiling of G-CSF responders in AML



Differential gene expression + markers from literature

Non-responders	Responders
CD14	CD46
CD32	CD69
CD36	CD117
CD86	CD133
CD93	CD155
CD11a	CD321
CD1d	
HLA-DR	

LSCs	
CD13	CD47
CD15	CD64
CD33	CD114
CD34	CD123
CD38	CD11b
CD44	CXCR4
CD45	TIM3



Screen pediatric AML samples
(18 diagnosis, 3 relapse, 7 healthy)

↓

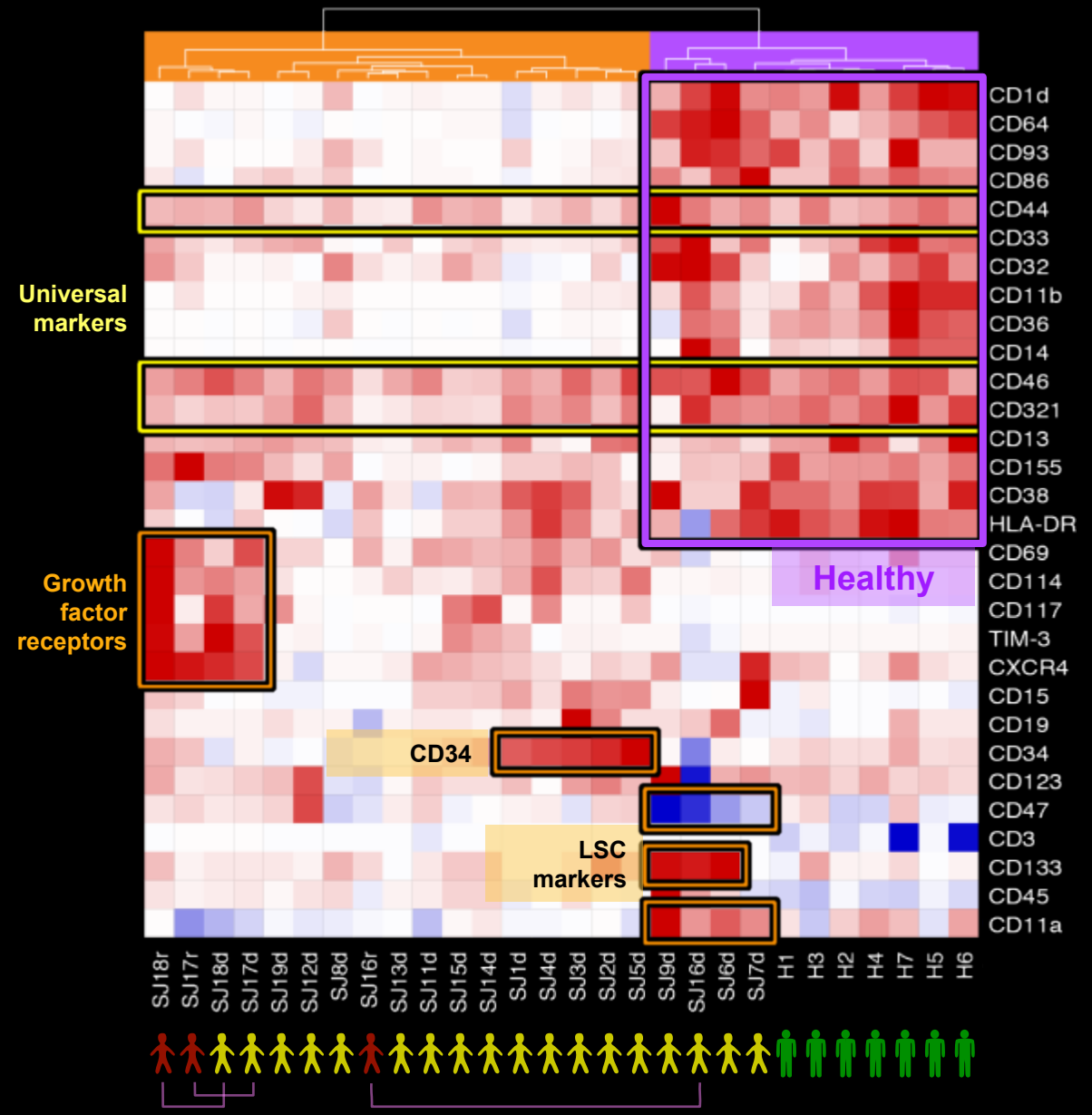
Gate G-CSF responders

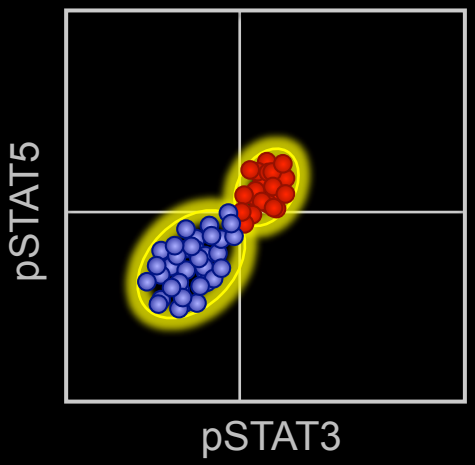
↓

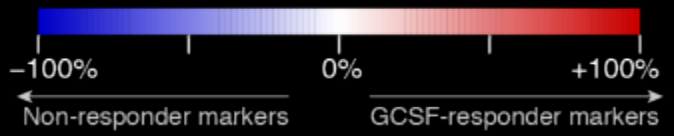
Identify enriched markers

with Faye Hsu, Yury Goltsev

Leukemic GCSF responders have distinct surface profiles







with Rob Bruggner & Sean Bendall

The cytometrist's toolbox

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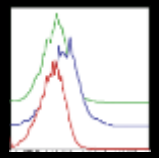
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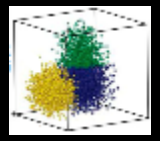
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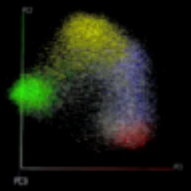
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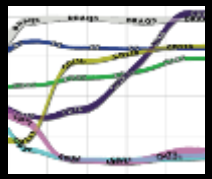
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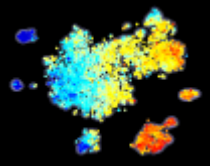
SPADE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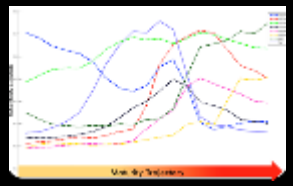
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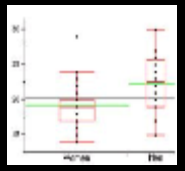
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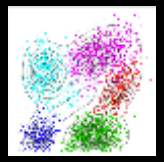
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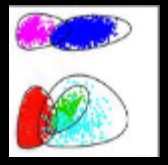
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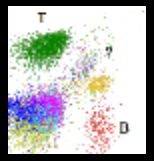
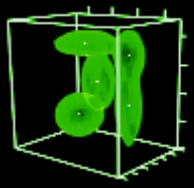
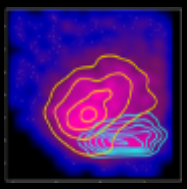
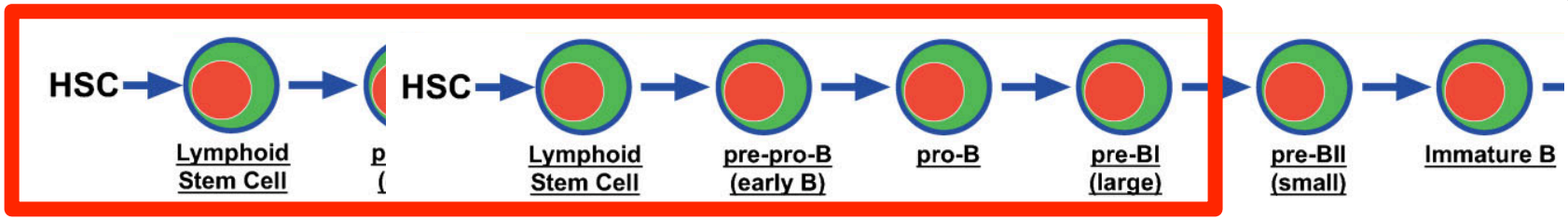

FLOCK


FLAME


SamSPECTRAL

Human B cell development is less understood than in the mouse



Surface Markers

CD34
CD38
CD10
IL7R
CD24
CD19

Intracellular Markers

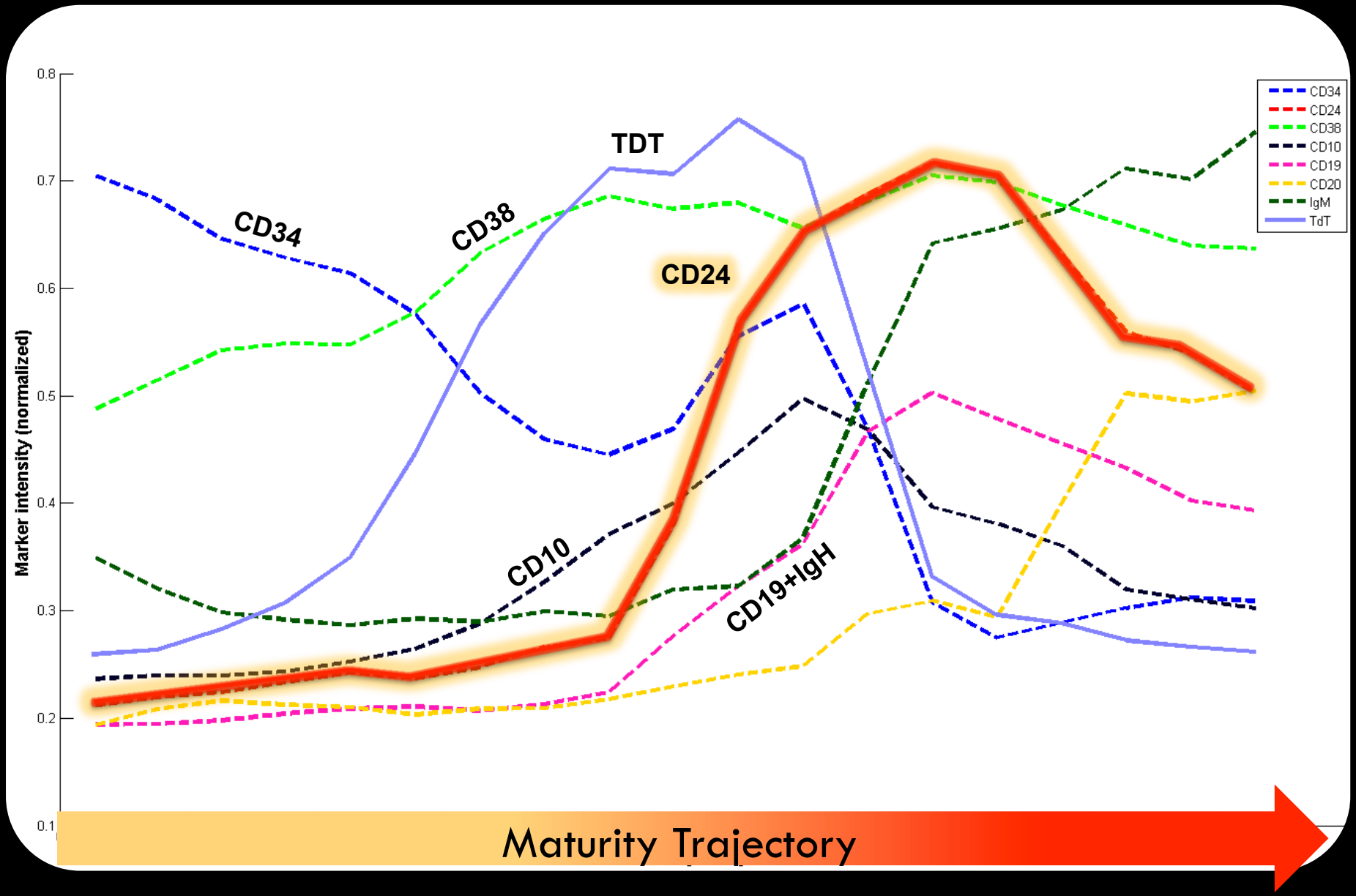
CD179a
Rag
TdT

IgH Genes

DJ_H
VDJ_H

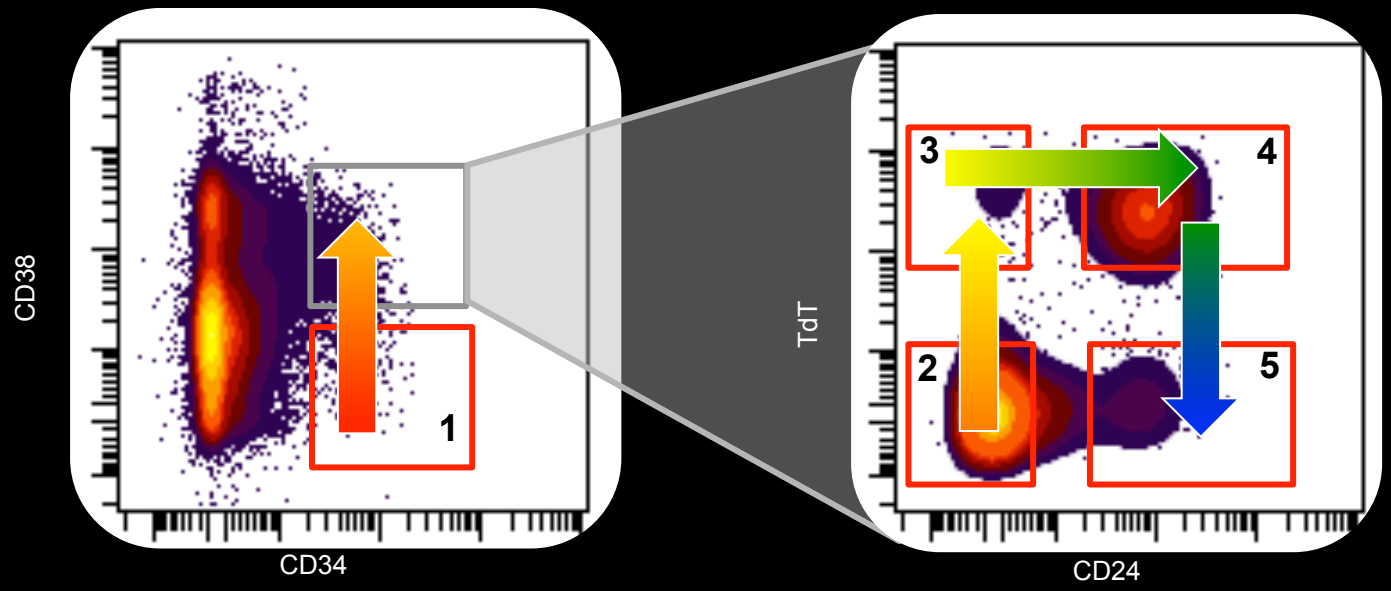
Adapted – Cobaleda, BioEssays 2009

The predicted trajectory ‘rediscovers’ human B cell development



“Wanderlust” algorithm: El-ad Amir, Dana Pe’er (Columbia)

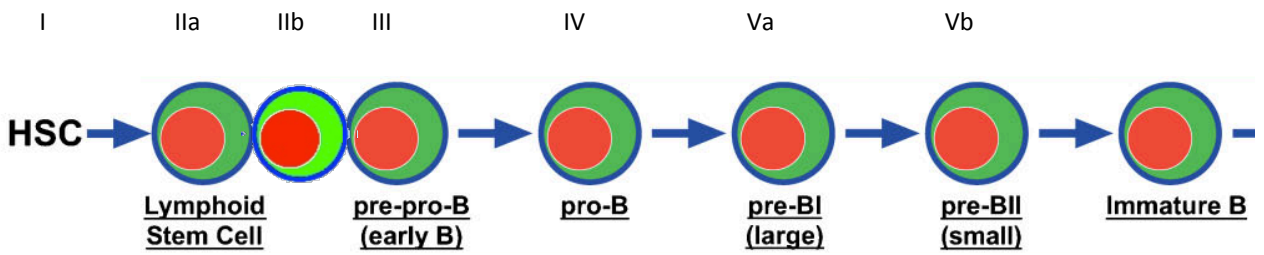
Following the predicted B cell trajectory



- Fluorescent panel design → Sorting → qPCR
- VDJ rearrangement confirms the trajectory:
  (unrearranged) 1 → 2 → 3 → 4 → 5 (rearranged)

Sean Bendall, Kara Davis

New insights and resolution in human B cell development



Surface Markers

CD34

CD38 ✓

CD10

IL7R

CD24

CD19 ✓

Cytoplasmic Markers

Ki67

CD179b

CD179a

Rag

TdT

pSTAT5

IgH Genes

DJ_H

VDJ_H

Sean Bendall, Kara Davis

Summary

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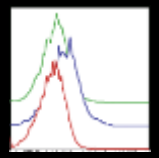
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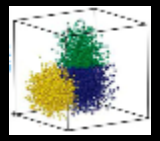
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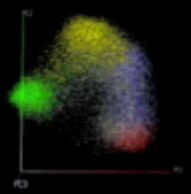
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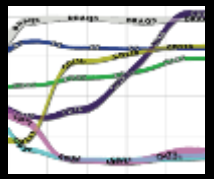
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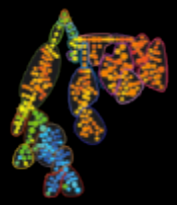
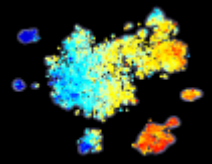

SPADE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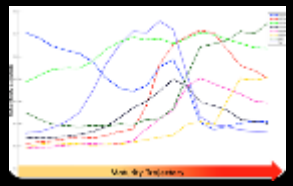
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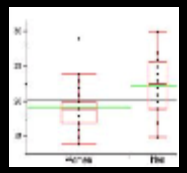
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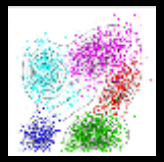
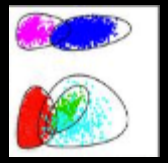

FlowClust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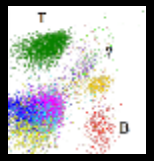
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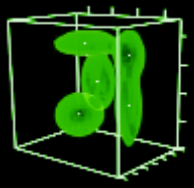
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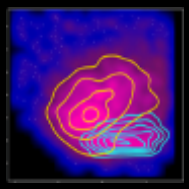
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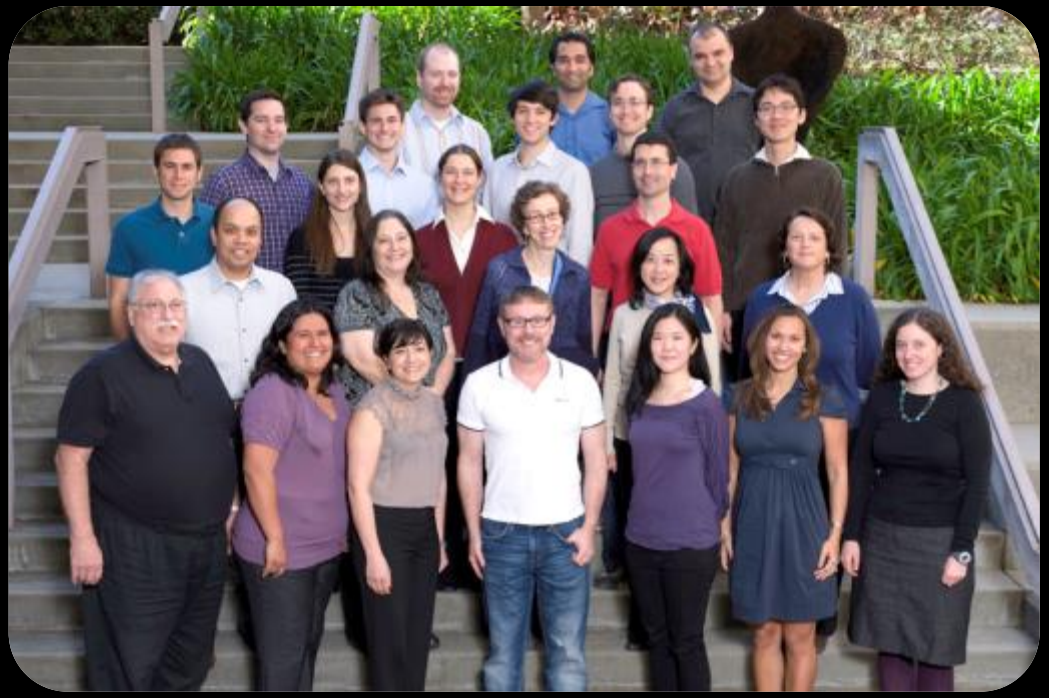

Prof. Garry P. Nolan

Sean Bendall

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Harris Fienberg
Astraea Jager

Rob Bruggner
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